The evolution of continuous flow elemental analyzer-IRMS combinations

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The implementation of continuous flow methodology into the fabric of isotope ration mass spectrometry (IRMS) is leading to significant evolution in sample preparation hardware for the determination of ^{13}C, ^{15}N, ^{18}O, ^{34}S and D of organic and inorganic samples. Between 1950, when the dual viscous flow inlet system was introduced, and the mid-1980s, only minor modifications were made to the hardware developed by Al Nier an co-workers. It was the mating of capillary gas chromatography (GC) with IRMS that allowed the dual inlet system to be bypassed, and the new arrangement has been widely referred to as "continuous flow-IRMS", because a carrier gas is used to achieve viscous flow conditions. At the present time, >50 % of all new IRMS systems are continuous flow-only instruments. The samples that are being analyzed by continuous flow methodologies include a wide variety of solids, liquids and gases, in which the concentration of the analyte of interest varies over a very large dynamic range. While there is little relaxation of the requirement for conversion of the analyte into a clean and pure gas prior to introduction into the ion source of the IRMS, the 'clean' gas is now a trace component in a stream of helium gas.

Quantitative High Temperature *Combustion:* The first known attempt to use an elemental analyzer with a mass spectrometer to measure phytoplankton uptake of enriched nitrogen dates back to 1965 (Barsdale and Dugdale, 1965), when a US group modified a Coleman nitrogen analyzer to combust the sample and CO_2 was used to sweep the nitrogen through the system. The CO_2 was absorbed in KOH solution and the N_2 cleaned over LN_2 and fed into a Bendix time-of-flight machine (serial #1). It is worth noting that this apparatus was even taken to sea. In 1983, two hybrid systems were constructed, with a Japanese group using small quadrupole mass spectrometer with a modified carbon-nitrogen elemental analyzer (Otsuki, A., *et al*., 1983), and an English group reporting on interfacing an automatic Carlo Erba elemental analyzer with an isotope ratio mass spectrometer (Preston and Owens, 1983). It is this last group that can be said to have invented the practice of "continuous flow-IRMS", which has led to the commissioning of over 600 EA-IRMS systems worldwide. This approach, which was originally focused on measurement of $\delta^{15}N$, was rapidly extended to include $\delta\ ^{13}C$ and $\delta\ ^{34}S$ (Pichlmayer and Blochberger, 1988) and more recently to $\delta\ ^{18}O$ and δ D (Hilkert, *et al*., 1999).

Combustion elemental analyzers perform a "flash combustion" of solid samples to CO_2, N_2, SO_2 and H_2O; the reaction products are entrained in a stream of helium (approx. 100 mL/min He), and either chemically trapped or separated on GC column. The commercially available elemental analyzers that are "continuous flow-IRMS compatible" are all based on the original instrument from Carlo Erba, whose innovation was the use of the GC column to separate the reaction products. The analyte peaks enter the mass spectrometer via an open split interface which performs a variety of tasks, including reduction of the flow rate down to 0.4 mL/min of He, dilution of large peaks to bring them within the dynamic range of the mass spectrometer, and insertion of pulses of reference gas into the He carrier stream. It is important to note that it is not only the sample inlet systems that have evolved; significant advances in collector technology allow novel measurement strategies including two components from a single reaction. Examples of such measurements include N_2 and CO_2 from an elemental analyzer combustion and CO and H_2 from a high temperature carbon reduction. The amount of information that can be gathered

from a single combustion is quite amazing, including δC, δN, atom% C and N, wt% C and N, and C/N. In general, C and N are analyzed together, and S is analyzed separately. The upper limit on C/N ratio that can be analyzed is determined by the ability of the interface to perform dilution on the larger peak (generally C) without fractionation; with the ConFlo III split interface, the dynamic range has been significantly extended (Werner *et al*., 1999). The lower limit of sample size is set partly by the blank and partly by minimum required signal intensity. Systematic work on methods of blank reduction (e.g. zero blank auto-samplers) and blank correction has led to ability to run sub-microgram samples of C and N.

Quantitative High Temperature *Reduction:* While the combustion elemental analyzer provided a practical solution to isotopic analysis of reduced carbon and nitrogen in a wide variety of solid materials, the isotopic analysis of oxygen and hydrogen proved to be a more difficult challenge, one which occupied many groups over a 30 year period, most notably for the analysis of $\delta^{18}O$ and δD in cellulose. The keys to successful analysis were the use of glassy carbon (Koziet, 1997) at elevated temperatures (>1450 ^{o}C) and (for $\delta^{18}O$) the analysis of the CO rather than CO_2, which lead to quantitative conversion from substrate to analytical species. A general term for this technique is "high temperature quantitative carbon reduction" (Douthitt, 2001). As with C and N from combustion EA, it is now possible to measure the isotopic composition of O and H from a single carbon reduction, as peaks of CO and H_2.

Dissolved organic carbon (DOC): The success of the EA-IRMS combination led us to examine whether any TOC analyzers used "continuous flow" and could be adapted to analysis of the isotopic composition of dissolved organic carbon (TOC or DOC). Successful coupling of a commercial TOC analyzer using high temperature chemical oxidation with the DELTA^{plus} was readily achieved. This combined DOC-IRMS combination can be used to determine total carbon mass and the isotopic composition of carbon, partitioned into TIC (total inorganic carbon) and TOC (total organic carbon, and achieves full automation of this formerly onerous manual analysis, and allows significant improvements in precision, accuracy, and throughput.

APPLICATIONS: The combination of EA-IRMS and TC/EA-IRMS allows total stable isotopic analysis of of biological materials- microgram-sized samples can be analyzed for δC, δN, δS, δO and δD, thus allowing true "isotope fingerprinting", which opens up the floodgates of applications which fall into the category of "applied isotope geology". While applications of isotopes to problems of fraud and adulteration in food and beverages are well known, a number of new areas of potential application are now opened. MIGRATION: Point of origin studies are closely related to studies of migration. The ability to use the stable isotopes (^{2}D, ^{18}O and ^{13}C) in animal or insect tissues to trace animal or insect movements across natural isotope gradients, particularly those in natural waters, was the subject of a recent conference. The source of the O and H in organic molecules is water, and if the isotopic composition of the organic molecules is archived in some fashion, it should be possible to read back a record "in isotope space" of the travels of an organism which is pertinent to the recent BSE concerns. POINT OF ORIGIN: Considerable ingenuity has been put into trying to determine "point of origin" for seizures of drugs of abuse, such as heroin, morphine and cocaine. It has been shown that the isotope fingerprinting can be used to differentiate at the 95% confidence level between heroin from each of the producing regions. OTHER POSSIBILITIES: Other applications of commercial interest include using ^{13}C and ^{15}N to characterize high explosives and ^{18}O, ^{13}C, and ^{2}H of cellulose as tools to investigate forgery of banknotes and commercial papers. The use of ^{13}C, ^{15}N and

potentially ^{2}H of amino acids in fixed tissues (e.g. fingernails) hold considerable potential as reliable monitors of common eating disorders as well as probes of metabolic states.

These new applications are nothing if not exercises in "applied isotopes". With a little imagination and some new analytical skills, isotope geochemists are better suited than most to exploit the considerable commercial opportunities afforded by these new capabilities which allow one to measure precisely and accurately the natural abundances of ^{2}H, ^{13}C, ^{15}N, and ^{18}O of individual organic compounds (CSIA), bulk materials (BSIA), and in some cases, specific positions on molecules (PSIA). It is our further observation that the expertise and involvement of isotope geochemists can be critical in helping new labs realize the full potential inherent in these new techniques.

Oxidation of organic C, N and organic and inorganic S by combustion to CO_2, N_2 and SO_2 is a relatively mature technique with a worldwide installed base of >600 EA-IRMS systems. The use of carbon reduction techniques for quantitative conversion of organic and inorganic O and H to CO and H_2 for bulk analysis is emerging rapidly (Sharp et al., in press), but with an installed based of 65 TC/EA is still in its infancy. Sample sizes for O measurements are in the same range of those in combustion mode but, because of the lower abundance of D and lower ionization efficiency of H_2, about 10 times more sample is required for D/H measurements. Quantitative carbon reduction of organic and inorganic O allows measurements hitherto difficult or impossible and thus offers considerable room for exploitation H.

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